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GRANT NUMBER DAMD17-98-1-8032

TITLE: Regulation of ErbB-2 and Src Signaling by CHK and Csk Tyrosine Kinases in Breast Cancer

PRINCIPAL INVESTIGATOR: Hava Avraham, Ph.D.

CONTRACTING ORGANIZATION: Beth Israel Deaconess Medical Center
Boston, Massachusetts 02215

REPORT DATE: May 1999

TYPE OF REPORT: Annual

PREPARED FOR: Commanding General
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	May 1999	Annual (23 Apr 98 - 22 Apr 99)

4. TITLE AND SUBTITLE
Regulation of ErbB-2 and Src Signaling by CHK and Csk Tyrosine Kinases in Breast Cancer

5. FUNDING NUMBERS
DAMD17-98-1-8032

6. AUTHOR(S)
Hava Avraham, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Beth Israel Deaconess Medical Center
Boston, Massachusetts 02215

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

Substantial evidence exists supporting direct roles for ErbB-2/neu and src tyrosine kinase activation in breast cancer. CHK is a kinase that phosphorylates and negatively regulates Src kinase activity.

Our studies reveal that CHK expression was observed in 70 out of 80 primary breast cancer specimens, but not in normal breast tissues (0/19). CHK participates in signaling in breast cancer cells by associating, *via* its SH2 domain, with ErbB-2 following heregulin stimulation. CHK-SH2 binds to Tyr1248 of human ErbB-2. Interestingly, autophosphorylation at this site confers oncogenicity to this receptor. Moreover, CHK was able to downregulate ErbB-2-activated Src kinases, and overexpression of CHK in MCF-7 breast cancer cells markedly inhibited cell growth and proliferative response to heregulin as well as decreased colony formation in soft agar. CHK also inhibits tumor growth of MCF-7 cells implanted in nude mice. These results strongly suggest that CHK is a potential novel negative growth regulator in human breast cancer.

New information gained from these studies on the role of CHK as a putative tumor suppressor gene may provide a basis for utilizing this novel tyrosine kinase to opppose the malignant process of breast cancer.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Breast Cancer	14
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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5/26/99

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Title: Regulation of ErbB-2 and Src signaling by CHK and Csk tyrosine kinases in breast cancer

P.I.: Hava Avraham, Ph.D.

INTRODUCTION

A major means by which Src kinases are downregulated is through C-terminal tyrosine phosphorylation. The Csk family of protein tyrosine kinases comprises two members termed Csk and CHK. These enzymes CHK (originally termed MATK) shares ~50% homology with the Csk tyrosine kinase. CHK is expressed in malignant breast tissue but not in normal breast tissue. In addition, CHK, in contrast to Csk, has the unique ability to bind via its SH2 domain to a particular diphosphorylated sequence (Tyr1248) on the C-terminus of the ErbB-2 receptor when activated by heregulin. Interestingly, this is the same sequence which confers oncogenicity to ErbB-2, suggesting a unique role for CHK in the regulation of ErbB-2 activation. Moreover, our preliminary results indicate that overexpression of CHK in MCF-7 breast cancer cells markedly diminished cell growth and inhibited tumor development of xenografts in nude mice. These results lead us to hypothesize that: (1) CHK may function as a negative regulator of both pp60src and ErbB-2, while Csk may function as a negative regulator of pp60src; (2) ErbB-2 activation results in the activation of Src kinases by their binding to an autophosphorylation site of ErbB-2, and that subsequent to this CHK binds to the Tyr1248 of the ErbB-2, resulting in phosphorylation and downregulation of the ErbB-2 and Src kinases; and (3) C-terminal phosphorylation of Src by Csk and CHK may be critical for downregulation of Src kinase activity and the inhibition of breast cancer growth. In order to test these hypotheses, we propose to focus on two basic aims: (a) To analyze the ability of CHK to downregulate ErbB-2 activated Src kinases; (b) To further characterize the effects of either CHK, Csk or both Csk and CHK, in preventing tumor development in tumor-bearing mice. New information gained from these studies in the role of CHK and Csk as putative negative growth regulators in breast cancer will advance current understating of oncogenic signal transduction mechanisms and may provide a basis for utilizing these tyrosine kinases to oppose the malignant process.

BODY

1. Expression of CHK in human breast cancer tissues: We have an ongoing collaboration in our studies on CHK in breast cancer with Dr. I. Keydar (Tel Aviv University, Ramat Aviv, Israel). We have access to more than 200 clinically characterized breast cancer cases for this study. In addition, we are able to obtain breast cancer specimens from the Cooperative Human Tissue Network (University of Alabama at Birmingham, Birmingham, Alabama). Most of the immunohistochemical studies were performed using specific CHK antibodies –48A (dilution 1:100). These antibodies were generated in rabbits immunized against the NH2-terminal of CHK (amino acid residues 22-16 of the CHK protein). Control antibodies such as preimmune 48A antiserum or absorbed 48A antiserum with the N-terminal peptide did not detect CHK expression in breast cancer specimens, indicating the specificity of CHK antiserum 48A. Furthermore, we have analyzed various anti-CHK anitsera, available in our lab as well as commercially available anti-CHK antibodies (anti-lsk, Santa Cruz Biotechnology). R-3662 antibodies against the CHK-SH2-SH3 domain and R-4110 antibodies against the C-terminus of CHK were raised in our lab. All these specific anitsera revealed specific staining of CHK in breast cancer specimens, while no expression was observed with the relevant preimmune sera (normal rabbit serum, normal IgG, preimmune R-3662 and preimmune R-4110), or with the absorbed antisera with either the

N-terminal-CHK peptide (for anti-lsk and 48A), the CHK-SH3-SH2 fusion protein (for R-3662), or the C-terminal-CHK peptide (for R-4110). Optimal immunohistochemical analyses for CHK staining have been achieved with antiserum 48A.

Analyses of CHK expression in human breast cancer tissues at different stages were performed using immunohistochemistry on paraffin sections. We assessed the level of CHK expression in breast tumors as compared to the adjacent normal tissues from the same patients. CHK protein expression was found in 70 out of 80 breast adenocarcinoma specimens (stage I – 32/41; stage II – 34/35; stage III – 4/4), but not in normal or benign breast tissues (normal and breast fibroadenoma – 0/19).

In addition, histochemical studies were performed using anti-CHK, anti Csk, and anti-ErbB-2, anti-estrogen receptor and anti-p53 antibodies.
We observed that in breast cancer tissues, there is an overexpression of CHK which correlates with (i) an overexpression of ErbB-2, (ii) a loss of estrogen receptor expression, and (iii) an increase of p53 expression.

2. Expression of CHK in normal and breast cancer tissues and cell lines: We examined a panel of normal breast tissues, breast cancer tumors, breast-carcinoma cell lines, normal epithelial cells (Hs578Bst and MCF10-A), and an SV40-transformed mammary epithelial cell line (HBL-100) for CHK expression using RT-PCR and Northern and Western blot analyses. While most of the breast carcinoma cell lines and tumors expressed CHK, no expression was found in the normal breast tissue, normal epithelial cells or HBL-100 cells as shown by RT-PCR (Fig. 1, Table 1). In agreement with the RT-PCR, Northern blot analysis of CHK expression in the carcinoma cell lines revealed different levels of expression of the CHK mRNA transcript of 2.3 kb, while no expression of CHK mRNA was observed in normal epithelial cells and HBL-100 cells (Table 1). Western blot analysis of CHK expression in these breast carcinoma cells also detected variable levels of CHK protein, in accordance with the Northern blot analysis, while CHK protein was not detected in normal breast tissues, normal epithelial cells or HBL-100 cells (see Table 1).

3. CHK expression in cancers and cell lines derived from non-breast tissues: We examined a panel of various cancers and carcinoma cell lines, with or without ErbB-2 amplification. CHK expression was not observed in most of these primary tumors or tumor cell lines as shown by immunohistochemistry and Western blot analyses (Table 2). However, expression of CHK was observed in ovarian tumors and ovarian tumor cell lines OVCAR-3 and SKOV-3, both overexpressing ErbB-2 (Fig. 2, Table 2).

4. Conditional ectopic expression of CHK in T47D cells: To conditionally express CHK protein in cell lines, we employed an artificial transcriptional regulatory system that uses the bacterially tetracyline resistant operator/repressor. Regulated, high level gene expression systems allow quantitative regulation of gene expression up to 1,000-fold induction in many cell types. The Tet-off system allows high level-regulated gene expression in response to varying concentrations of tetracyline (Tc). In this system, gene expression is turned on in the absence of Tc. The Tet expression system is based on two regulatory elements derived from the tetracycline resistance operon of the *E. coli* Tn 10 transposon: the tetracycline repressor protein (Tet R) and the tetracycline operator sequence (Tet O) to which Tet R binds. Flag-CHK cDNA was subcloned into the expression vector PUHD10-3, downstream of a synthetic promoter composed of tandem repeats of the tetracyline operator, and a CMV promoter as described (Fig. 3A) (Clontech Laboratories, CA). Activation of this promoter is dependent on the co-expression of an artificial transactivator composed of the tetracycline repressor fused to the activation domain of the herpes simplex virus transcriptional activator V16. The presence of tetracycline prevents the binding of this transactivator to the operator and therefore inhibits the

expression of Flag-CHK mRNA. Thus, CHK is expressed in the absence of tetracycline; however, in the presence of tetracycline, CHK is not expressed.

The plasmid, PUHD15-1 expressing transactivator, was transfected with the Flag-CHK expression vector and a neomycin-resistant plasmid, pSV2neo, into T47D cells. Conditional expression of CHK mRNA in stably transfected cells was assessed by Northern and Western analyses of total mRNA or total cell lysates, respectively, isolated from G418-resistant colonies cultured in the presence or absence of tetracyline. The Northern blot analysis showed no CHK mRNA expression in single T47D cell clones grown in the presence of tetracycline. Removal of the drug resulted in an induction of the exogenous mRNA over a 48 h period. Western blot analysis did show CHK protein expression in T47D cell clones in the absence of tetracyline. The level of CHK expression was comparable to that of MCF-7 cells stably transfected with pcDNA3neo/CHK (clone 10).

Four stable cell lines were generated, T1S1, T1S2, T1S3 and T1S4, that express CHK mRNA and protein in a tightly controlled tetracycline dose-dependent manner (Fig. 3). Overexpression of CHK in these clones was not toxic, as determined by counting viable cells and by MTT assay. CHK expression was dose-dependent on tetracyline (Fig. 3C). The increase in CHK expression could be reversed by adding tetracycline to the culture medium of the cells. In addition, our preliminary data indicated that under conditions of serum deprivation for 1, 2, or 3 days, there was no induction of apoptotic cell death, as analyzed by light microscopy, flow cytometry, and DNA gel electrophoresis. Therefore, these preliminary data suggest that overexpression of CHK does not result in toxicity or induction of apoptosis.

Tight and reversible regulation of CHK expression in stable cell clones will allow us to gain insight into the biological function(s) of CHK in normal and malignant mammary epithelial cells, and avoid the dramatic variability in biological response(s) to ectopically expressed proteins as seen in individually selected clones with other transfection systems. Future studies will address CHK function using these clones.

5. Effect on cell proliferation and colony formation: We generated stable transfected cells overexpressing CHK, either wild-type or kinase-dead. The proliferation rate of MCF-7/CHK[wild-type] cells was significantly reduced compared to untransfected MCF-7 cells or MCF-7/CHK[kinase-type] cells. The number of colonies formed in soft agar by MCF-7/CHK[wild-type] cells decreased approximately 4-fold compared to untransfected MCF-7 cells and MCF-7/CHK[kinase-type] cells.

6. Tumor development in nude mice: Our studies have shown that CHK negatively regulates src activity and associates with ErbB-2 upon heregulin stimulation. CHK can reduce the proliferative activity of breast cancer cells and cause desensitization to the growth-promoting effects of heregulin. Furthermore, overexpression of CHK in MCF-7 breast cancer cells decreased colony formation in soft agar. Therefore, CHK might function as a negative regulator and might act to inhibit mitogenic signaling mediated b c-src and ErbB-2. To evaluate the anti-transforming potential of CHK, we monitored tumor development in nude mice injected with MCF-7 breast cancer cells, or MCF-7 cells stably transfected with either the pcDNA3neo vector (MCF-7/neo), or the pcDNA3neo/CHK construct overexpressing CHK protein (MCF-7/CHK cells). Female athymic nude mice of 4- to 6-week old Balb/c nu/nu were maintained in microisolator cages within a pathogen-free isolation facility. The mice were anesthetized with pentobarbital sodium, and the MCF-7 cells or the MCF-7 stably transfected clones (1×10^6 in 0.05 ml of culture medium) were resuspended in 100 µl of Matrigel (Collaborative Biomedical Products, Bedford, MA) and injected to mammary fat pad tumors were measured twice each week, and the tumor volume was calculated as the sum of the tumor volumes divided by the number of tumors. Tumor development in nude mice injected with MCF-7/CHK cells (clones 5 and 10) was significantly reduced compared to tumor development in nude mice injected with the control MCF-7

cells or MCF-7/neo cells. These preliminary experiments suggest that overexpression of CHK can negatively regulate the growth of MCF-7 breast cancer cells in nude mice.

7. **Sequence analysis of CHK cDNAs in breast cancer:** CHK is expressed in primary breast tumors, but not in normal breast epithelial cells. To determine whether the CHK gene expressed in breast cancer cells is a normal or mutated form, we isolated CHK cDNA clones from four different primary breast tumors using RT-PCR analysis. These breast tumors were found to express CHK as shown by immunohistochemistry. Briefly, poly (A)+ RNA was isolated from four different primary breast tumors that were found to express CHK based on immunohistochemical staining. CHK sequences were amplified with degenerate oligonucleotide primers. PCR products of the amplified CHK were purified from the agarose gel, ligated into a TA vector and transformed in E. *coli* DH5α. Sequencing was carried out and was compared to wild-type CHK cDNA isolated from megakaryocytes and brain. A total of 16 clones were isolated and sequenced. Sequence analyses of these clones indicated that there were no mutations in the CHK cDNAs expressed in these breast tumor samples. These results might suggest that: (1) the phosphorylating ability of the endogenous CHK expressed in breast cancer is altered compared to CHK in normal hematopoietic cells and brain; (2) additional functional isoforms or a non-functional form of CHK exists in breast cancers; and (3) the CHK expression observed in breast tumors and not in normal breast epithelial cells could be due to transcriptional regulation of CHK expression during breast tumor development.

KEY RESEARCH ACCOMPLISHMENTS:

Our studies demonstrate that CHK downregulated Src kinases activated by heregulin.

- CHK expression is upregulated in breast cancers and overexpression of CHK inhibits tumor formation in breast cancers grafted in nude mice.

REPORTABLE OUTCOMES:

We have submitted an abstract that was presented at the meeting of AACR in April '99.

CONCLUSIONS:

A. Overexpression of CHK correlates with the known markers of breast malignancy.
B. Overexpression of CHK can negatively regulate the growth of MCF-7 breast cancer cells in nude mice.
C. These results suggest that CHK overexpression is associated with anti-proliferative activity and can reduce the transformation ability of breast cancer cells.

BIBLIOGRAPHY

We have submitted an abstract that was presented at the meeting of AACR in April '99.

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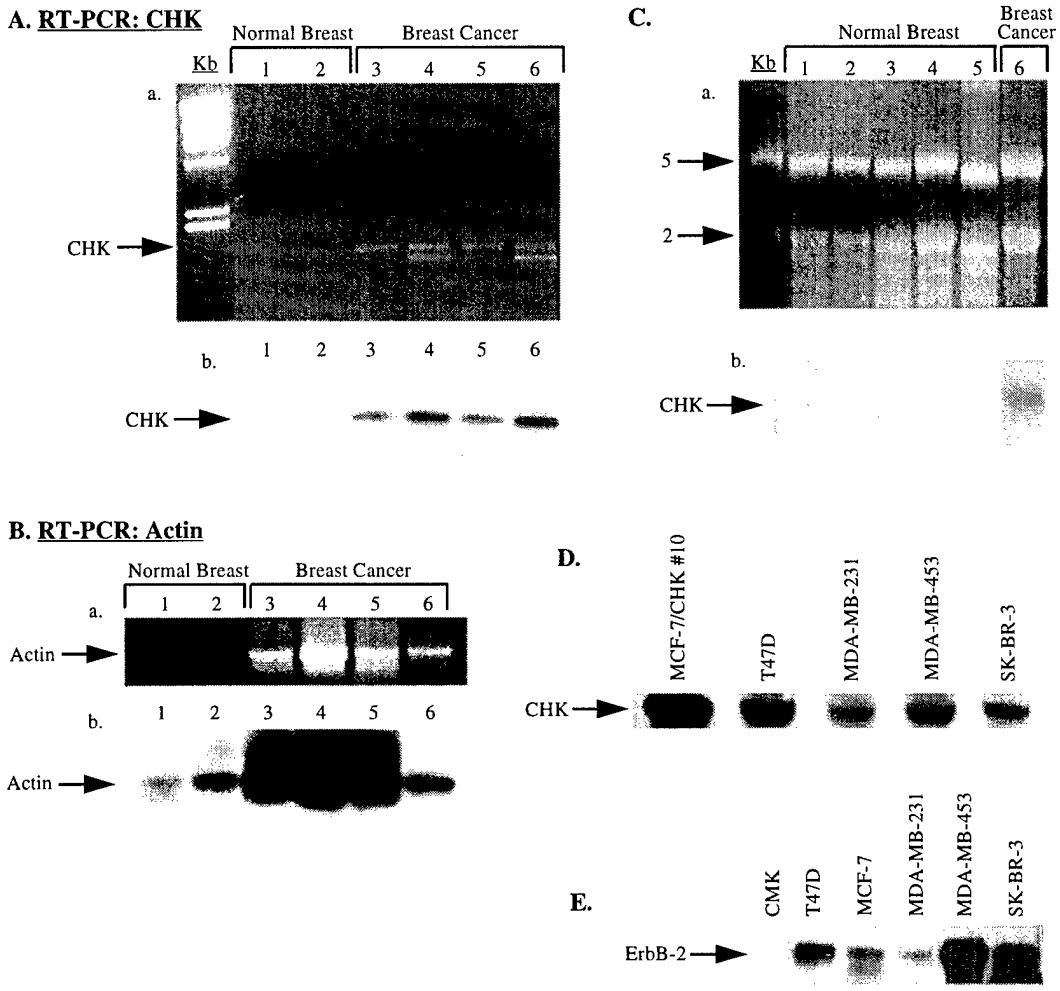

Figure 1: Expression of CHK in normal human breast specimens and human breast cancer tumors

This is a representative analysis of CHK expression in breast tumors and cell lines. CHK expression by RT-PCR in two normal breast specimens and four breast cancer tumors. Poly (A+) RNA was extracted from each sample and PCR products were obtained using CHK-specific primers (panel A) or actin-specific primers (panel B), as described previously in our work (Bennett et al., J. Biol. Chem., 269:1068-1074, 1994). The PCR products were electrophoresed on a 2% agarose gel and hybridized with full-length CHK cDNA (panel A-b) or actin cDNA (panel B-b) specific probes.

(C) CHK expression by Northern blot analysis. CHK expression by Northern blot analysis in normal breast specimens (samples 1-5) and breast cancer tissue (sample 6). Poly (A+) RNA (2 μg) was extracted from each tissue, electrophoresed in a denatured 1% agarose-formaldehyde gel, and transferred to a nitrocellulose filter. The filters were hybridized with ^{32}P-labeled CHK cDNA. CHK expression was observed only in breast cancer tumor sample 6, and not in normal breast tissues.

(D) Expression of CHK in breast cancer cell lines by Western blot analysis. Total cell lysates from each cell line was prepared (200 micrograms for each cell line) and analyzed for CHK expression by Western blot analysis using anti-CHK antibody.

(E) Expression of ErbB-2 in breast cancer cell lines by Western blot analysis. Total cell lysates from each cell line (200 micrograms) were prepared and analyzed for ErbB-2 expression by Western blot analysis using anti-ErbB-2 antibody.

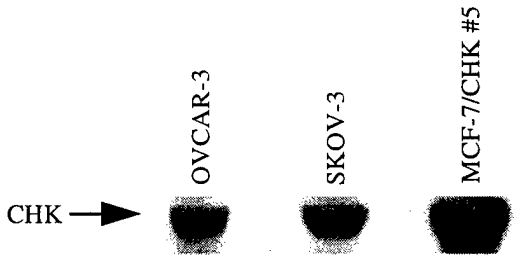

Figure 2: Expression of CHK in ovarian carcinoma cell lines
Ovary carcinoma cell lines (OVCAR-3 and SKOV-3) were analyzed for CHK expression by Western blot analysis and compared to stably transfected MCF-7 cells overexpressing CHK, MCF-7/CHK #5. Two hundred micrograms of total cell lysates were prepared from each cell line and were examined by Western blot analysis using anti-CHK antibody.

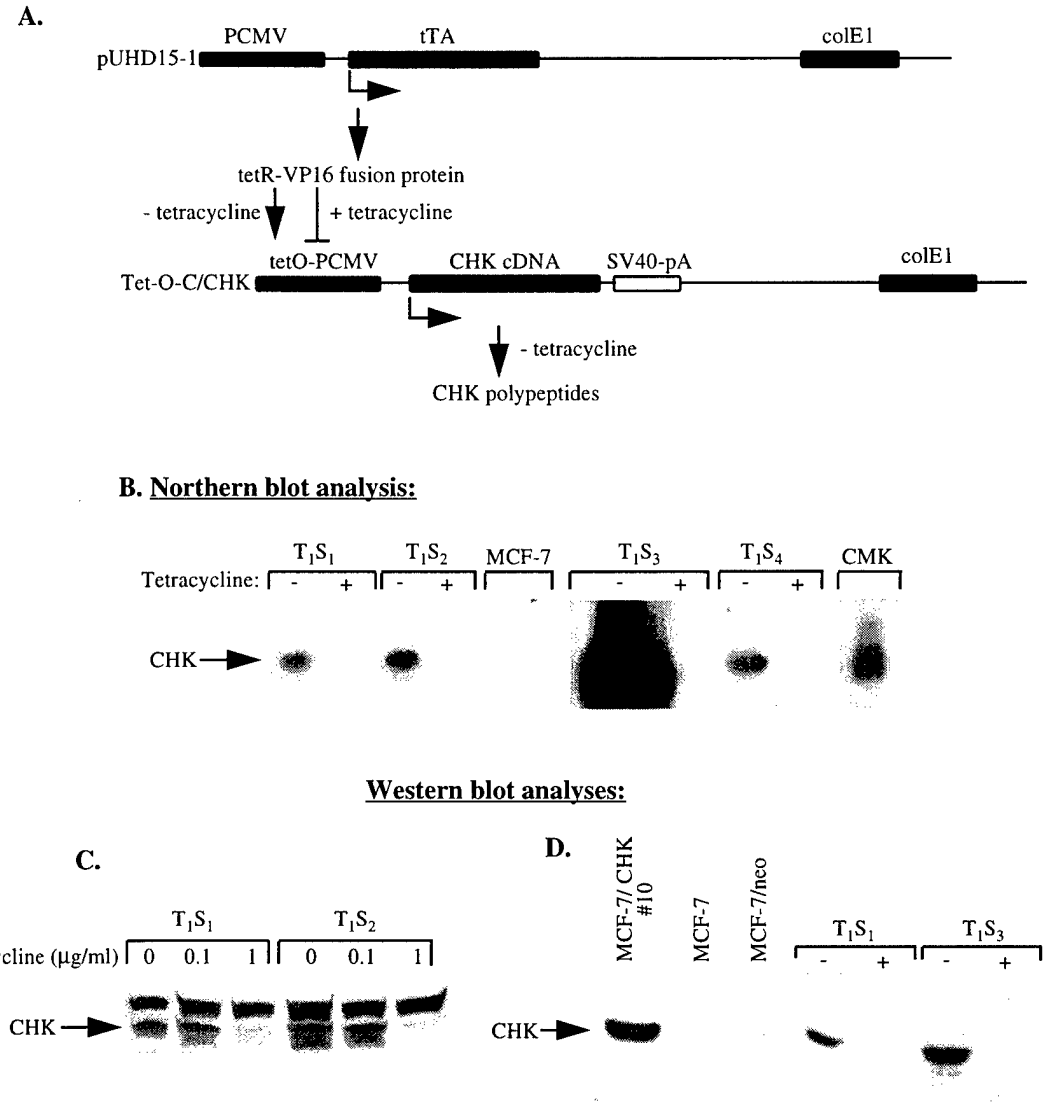

Figure 3: Inducible expression of CHK in T47D cells

(A) An illustration of the tetracycline activator expression system.

(B) Induction of CHK mRNA expression in selected transfected T47D clones in response to removal of tetracycline. Cells were maintained in growth medium containing 1 μg/ml of tetracycline. When the cells reached ~50% confluence, cells were split and either continued to grow in the presence of tetracycline (+) or tetracycline was removed and the cultures were incubated for 48 h. Forty micrograms of total RNA isolated from each sample were analyzed by Northern blot hybridization for expression of the CHK mRNA. mRNA isolated from the CMK megakaryocytic cell line served as a positive control.

(C) Western blot analysis of CHK protein expression in two selected clones T_1S_1 and T_1S_2 in the presence or absence of tetracycline (0, 0.1 or 1 μg/ml) for 48 h. Forty micrograms of total cell lysates were prepared from both cell lines and examined by Western blot analysis using anti-CHK antibody.

(D) Western blot analysis of CHK expression in MCF-7 and stably transfected MCF-7 clones (MCF-7/neo and MCF-7/CHK clone 10) compared to ectopic expression of CHK in T47D transfected clones (T_1S_1 and T_1S_3). Forty micrograms of total cell lysates were prepared from each clone and examined by Western blot analysis using anti-CHK antibody.

Table 1: Expression of CHK in normal and breast cancer tissues and cell lines
CHK expression by RT-PCR analysis: The PCR products were electrophoresed on a 2% agarose gel and hybridized with full-length CHK cDNA as a probe.
Expression was determined based on hybridization with CHK cDNA.
CHK expression by Northern blot analysis: Poly (A+) RNA was isolated from breast tissue and cell lines as indicated and was analyzed by Northern blot using CHK cDNA as a probe.
Plus (+) symbol indicates hybridization and minus (-) symbol indicates no hybridization detected.
CHK expression by Western blot analysis: Total cell lysates were prepared from breast tissues and cell lines and were analyzed for CHK expression by Western blot analysis.

Breast carcinoma cell lines	**RT-PCR**	**NB**	**WB**
MCF-7	+	-	-
T47D	+	+	+
MDA-MB-231	+	+	+
MDA-MB-453	+	+	+
SK-BR-3	+	+	+
Normal epithelial cell lines			
Hs378Bst	-	-	-
MCF-10A	-	-	-
HBL-100	-	-	-
Normal breast tissues			
10 specimens	0/10	0/10	0/10
Primary breast tumors			
12 specimens	12/12	10/12	10/12

Table 2: CHK expression in primary cancer tissues and cancer cell lines
Immunohistochemical staining was performed on paraffin embedded sections of various tumors using anti-CHK or anti-ErbB-2 specific antibodies.

Type of cancer	Immunohistochemistry analysis	
	CHK	ErbB-2
Multiocular cystic renal carcinoma	-	-
Bronchioalveolar carcinoma	-	-
Liver-moderately differentiated adenocarcinoma	-	-
Highly malignant glial neoplasm	+	+
Cystic renal cell carcinoma	-	-
Differentiated ductal carcinoma	-	-
Colon carcinoma	-	-
Ovarian carcinoma	+	++
Ovarian carcinoma	+	++

Total cell lysates were prepared from cells (5 x 10^6/ml) and were analyzed by 7.5% SDS-PAGE, transferred onto membrane and subjected to Western blotting using specific antibodies for CHK and ErbB-2 as described.

Cancer cell lines	Expression by Western blot	
	CHK	ErbB-2
Ovarian tumor cell line OVCAR-3	+	++
Ovarian tumor cell line SKOV-3	+	++
Bladder carcinoma 5637	-	-
Cervix carcinoma CaSki	-	-
Colon carcinoma COLO201	-	-
Kidney carcinoma 769-P	-	-
Liver carcinoma Capan-1	-	-
Lung carcinoma A-427	-	-

is also downregulated in breast cancer. By using the breast carcinoma cell line BT-474 and an RT-PCR method for zyme, we have found that this gene is upregulated by estrogens and, possibly, progestins.

#231 Identification of novel isoforms of human RAD52 gene involved in DNA double-strand breaks (DSBs) repair. Kito, K., Kamitani, T., Nezu, K., Abe, Y., Sugita, A., Wada, H., Yeh, T.H.E., and Ueda, N. *Department of Pathology, Ehime University School of Medicine, Shitsukawa, Shigenobu, Onsen-gun, Ehime 791-0295 JAPAN; and Department of Molecular Medicine, The University of Texas, 6431 Fannin, Houston, TX 77030.*

RAD52 gene is involved in double-strand breaks (DSBs) in DNA induced by ionizing radiation or various chemical agents. In yeast, RAD52 gene has been shown to be essential for DSBs repair through homologous recombination pathway. Recently human homologue of the yeast RAD52 gene which encoded 418 amino acid residues has been identified. In this study, we present three distinct but related isoforms of human RAD52 isolated from brain and testis cDNA libraries. All isoforms contain different inserts that cause translational frame-shifts producing truncated proteins. The three isoforms consist of 226, 139 and 118 amino acid residues, we tentatively designated them as RAD52β, γ and δ, respectively. We detected messages of the isoforms in a number of human tissues, notably in ovary, testis and small intestine. As interesting characteristics of RAD52, it has been demonstrated that RAD52 protein interacts with itself, and also binds to both single-stranded (ss) and double-stranded (ds) DNA to promote homologous recombination. Here we demonstrated that they could bind to both ss- and dsDNA like RAD52 protein although they could not interact with RAD52 proteins. These data suggest that RAD52β, γ and δ may participate in DNA recombination/repair pathway through their DNA binding property and may contribute to a dominant negative effect as the result of competing in DNA binding sites.

#232 Functional characterization of CHK as a novel negative growth regulator of human breast cancer. Bougeret, C., Deng, B., Keydar, I., and Avraham, H. *Division of Experimental Medicine, Beth Israel Deaconess Medical Center, Harvard Institutes of Medicine, Boston, MA 02115 (C.B., B.D., H.A.); and Department of Cell Research and Immunology, Tel Aviv University, Ramat Aviv 69978, Israel (I.K.).*

We previously demonstrated that CHK participates in signaling in breast cancer cells by associating, *via* its SH2 domain, with Tyr1248 of the human ErbB-2 receptor following heregulin stimulation. Moreover, CHK is able to downregulate ErbB-2-activated Src kinases. In this study, we further evaluated the function of CHK as a novel negative growth regulator of human breast cancer. We observed that CHK is expressed in human primary breast cancer tissues (70/80), but is not detected in normal or benign breast tissues (0/19). In addition, we observed a co-localization of CHK and ErbB-2 in human breast cancer specimens (12/12), as demonstrated by confocal microscopy. To evaluate the anti-transforming potential of CHK, we monitored tumor development of human breast cancer cells grafted in nude mice. We implanted MCF-7 cells either untransfected or transfected with CHK. The tumor growth of MCF-7/CHK cells (n=10) was compared to that of untransfected MCF-7 cells (n=10), using a two-tailed Mann-Whitney non-parametric rank test. The tumor volume (measured after 7 weeks) of MCF-7/CHK cells was significantly reduced ($p < 0.05$) compared to that of the untransfected MCF-7 cells. This suggests that overexpression of CHK can negatively regulate the growth of MCF-7 cells in nude mice. Our data strongly support the role of CHK as a new tumor suppressor gene, presumably through downregulation of Src and/or ErbB-2 kinase activity, and provide a basis for utilizing this novel tyrosine kinase to oppose the malignant process of breast cancer.

#233 The role for NES1 serine protease as a novel tumor suppressor. Goyal, J., Smith, K.M., Cowan, J.M., Wazer, D.E., Lee, S.W., Band, V. *Departments of Radiation Oncology [J.G., D.E.W., V.B.], and Pathology [J.M.C.], New England Medical Center, and Department of Biochemistry [V.B.], and Genetics Program [K.M.S., V.B.], Tufts University School of Medicine, Boston, Massachusetts 02111, and Department of Gerontology, Beth Isreal Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts 02115 [S.W.L.]*

Previously, we isolated a novel serine protease-like gene NES1 (Normal Epithelial Cell Specific-1) that is expressed in normal mammary epithelial cells but is down-regulated in most breast cancer cell lines. Here, we demonstrate that stable expression of NES1 in the NES1-negative MDA-MB-231 breast cancer cell line suppressed the oncogenicity as revealed by inhibition of the anchorage-independent growth and tumor formation in nude mice. Fluorescence in-situ hybridization localized the NES1 gene to chromosome 19q13.3, a region that contains genes for related proteases including the prostate specific antigen, and is rearranged in human cancers. Similar to breast cancer cell lines, prostate cancer cell lines also lacked NES1 mRNA and protein expression. Together, these results strongly suggest a tumor suppressor role for NES1 in breast and prostate cancer.

#234 The ubiquitin carboxy-terminal hydrolase, BAP1, is a novel tumor suppressor gene. Jensen, D.E., Proctor, M., Chernova, T., Wilkinson, K.D., Minna, J., and Rauscher, F.J. III. *The Wistar Institute, Philadelphia, PA 19104; The Hamon Center for Therapeutic Oncology Research, University of Texas Southwestern Medical Center, Dallas, TX 75235; and the Department of Biochemistry, Emory University, Atlanta, GA 30322.*

BAP1 is the only nuclear-localized member of the ubiquitin carboxy-terminal hydrolase (UCH) family of enzymes. BAP1 binds to the wild-type BRCA1 RING finger domain, but not to mutated BRCA1 RING finger domains, suggesting that the loss of this interaction can lead to cancer. The *BAP1* gene is located at chromosome 3p21.3 and we have detected rearrangements, homozygous deletions and missense mutations within the *BAP1* gene in lung cancer cell lines and breast tumor samples. The missense mutations that were identified in the cell lines (A95D and G178V), and which occur within the catalytic domain of BAP1, show reduced enzymatic activity, suggesting that this activity is crucial to proper cell growth. Indeed, stable expression of BAP1 in a cell line lacking endogenous BAP1 reduced the growth rate of these cells and affected cell morphology. Expression of the BAP1 (A95D) or the BAP1 (G178V) mutants showed growth rates similar to, or slightly slower than, vector controls. Analysis of the stable cell lines for growth in suspended medium (soft agar) showed that BAP1 significantly reduced the size and number of colonies formed compared with controls. These data support a hypothesis that *BAP1* is a novel tumor suppressor gene and suggests the possibility that mutations in the *BAP1* gene may account for some familial-derived breast tumors which lack BRCA1 mutations.

#235 Studies on three novel human colorectal cancer related genes. Zheng, S., Cao, J., Cai, X.H., Shi, Z.Z., Zheng, L., Geng, L.Y., Zhang, Y.M., Zhang, S.Z., Mo, Y.Q. and Cao, W. *Cancer Institute, Chinese Academy of Medical Science, Zhejiang Branch. Hang Zhou, Zhejiang 310009, P.R. China.*

In order to understand the molecular mechanisms of colorectal cancer, the subtractive hybridization between cDNA of normal colon mucosa and mRNA of colorectal cancer (CRC) tissue was performed. A total of 46 clones, which down-regulated in colorectal cancer, were isolated and the sequence homology analysis were performed against the Genbank database. Three out of 46 cDNA clones (SNC6, SNC19, SNC73) have been studied, and their sequences have been accepted as new genes by NCBI. Some characteristics of these three colorectal cancer related genes are summarized in table 1.

Table 1: Characteristics of three new colorectal cancer related genes

	Genebank Accession	CDNA Size(bp)	ORF (AA)	Chromosome Localization	Low Expression Rate in CRC*
SNC 6	U17714	3168	369	22q13	32–60%
SNC 19	U20248	2900	ND	11q24	ND
SNC 73	AF067420	1610	384	14q32	53–80%

* By Northern blot, Dot blot and RT-PCR

LOH has been frequently detected in chromosomal regions of SNC6 and SNC19 in colorectal cancer, breast cancer and ovarian cancer. Retroviral transfer of these genes into colorectal cancer cell line SW1116 leads to the growth inhibition and decreased colony formation in soft agar. Our data suggested that SNC6, SNC19 as well as SNC73 are candidate tumor suppressor genes and may play important role in the colorectal cancer tumorigenesis.

#236 SEC14L maps to a discrete region of loss on 17q25 in breast and ovarian tumors and may function as a tumor suppressor. Kalikin L.M., Bugeaud E.M., Sims H.L., and Petty E.M. *Departments of Internal Medicine and Human Genetics, University of Michigan, Ann Arbor, MI 48109-0638.*

Although laudable progress has been made in characterizing several genes involved in breast and ovarian cancer, additional genes are likely involved in the malignant transformation of mammary and ovarian epithelial cells. We previously mapped a putative tumor suppressor gene to a 300 kb interval on human chromosome 17q25 in primary breast and ovarian tumors by allelic imbalance studies. We constructed a contig spanning the interval and mapped approximately 50 genes and ESTs in relationship to our contig. Only one gene, SEC14L, mapped precisely within the candidate interval. SEC14L has homology to a yeast secretory protein and to a squid retinal-binding protein. As several recent studies suggest that retinoic acid (RA) inhibits proliferation of breast cancer cells, loss of function of a gene with retinal binding properties may serve to augment the progression of mammary tumorigenesis. To explore the potential role of SEC14L in breast cancer we initially performed Northern blot analysis of 21 breast cancer cell lines and detected a transcript variant in one cell line. Interestingly, the proliferative affects of RA in this cell line demonstrate that its growth rate is unaffected by the presence of RA while a control breast cancer cell line displayed a significantly reduced growth rate. No sequence variants in the coding regions of this aberrant transcript have been identified. Further expression, mutational, and functional studies will help determine the significance of SEC14L in breast and ovarian cancer.